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Final Report

Assessment of Parasites and Chalkbrood Disease in Saskatchewan Alfalfa Leafcutting Bee Populations, and Development of Control Methods

R-92-CA-0739
92000053

Funded by
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9200053

SASKATCHEWAN ALFALFA SEED PRODUCERS' ASSOCIATION

FINAL RESEARCH REPORT

ADF PROJECT R-92-CA-0739

Assessment of parasites and chalkbrood disease in Saskatchewan alfalfa leafcutting bee populations, and development of control methods

March 31, 1994

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A. Executive Summary

TITLE : **Assessment of parasites and chalkbrood disease in Saskatchewan alfalfa leafcutting bee populations, and development of control methods.**

PROJECT NO. : **R-92-CA-0739**
Saskatchewan Agriculture Development Fund

I. Abstract

Surveys of Saskatchewan leafcutting bee populations in 1992 and 1993 have identified a significant outbreak of chalkbrood disease (Ascosphaera aggregata) in one population. Parasite levels have decreased in many populations due to cool, wet weather in the past two field seasons. Research on dichlorvos, currently used for parasite control, has demonstrated that rate, treatment time, number of bees treated, position of bees in the incubator, and incubation humidity may be implicated in dichlorvos-induced bee mortality. Promising alternative compounds for parasite control have been identified and their efficacy demonstrated. Research has led to registration of paraformaldehyde for bee cell fumigation and has demonstrated its efficacy for chalkbrood disease control.

II. Non-technical summary of results and impact

Surveys of Saskatchewan leafcutting bee populations from the 1992 and 1993 field seasons have identified a significant outbreak of chalkbrood disease (Ascosphaera aggregata) in a population in the northeast area of the province. This economically damaging fungal disease causes high mortality in leafcutting bee populations in northwestern United States and in southern Alberta and poses a serious threat to Saskatchewan leafcutting bee populations. Other fungal and bacterial species, referred to as leafcutting bee microflora, are present at higher levels in bee populations. Annual surveys have also indicated decreased parasitism in 1992 and 1993 surveys, due largely to the cool, wet conditions experienced during July and August the past two years. These conditions have also caused a decrease in leafcutting bee reproduction and have created an ideal environment for the increased growth of microflora in leafcutting bee populations.

Research to examine the effect of dichlorvos use for parasite control at various rates over several time periods has shown that bee mortality increased with increasing dichlorvos rates and with early dichlorvos treatment. Further research on the effect of dichlorvos in relation to the number of bee cells treated indicated that bee mortality was lower in tests with larger numbers of bees, indicating the importance of a minimum critical number of bee cells per unit area to normal bee development. Work to determine the effect of various dichlorvos treatments in relation to cell position in incubation trays indicated that bee mortality in cells from the top layer of trays was substantially higher than mortality in cells sampled randomly from throughout the trays; mortality was due to increased dichlorvos exposure.

Research to elucidate the mechanism at work in the area of dichlorvos and its effects on leafcutting bee development has also examined the effect of relative humidity in the incubator during dichlorvos treatment for parasite control. The effect of relative humidity on dichlorvos-induced leafcutting bee mortality was significant in the low to moderate humidities examined. Adult emergence from cells treated with dichlorvos at the recommended rate was significantly decreased at incubation humidities of 28% and 59%. Dichlorvos-induced mortality in developing leafcutting bee pupae is apparently increased with lower humidities due to increased dichlorvos release in a low humidity atmosphere and due to increased dichlorvos uptake by the developing bee pupa across the bee cell surface. Alfalfa seed producers incubate leafcutting bees at various relative humidities, and incubation humidity is often affected by ambient conditions (i.e. wet or dry spring/summer). As noted above, dichlorvos rate, treatment time, number of bees treated, and position of bees in the incubation tray may all significantly affect bee emergence. Relative humidity is an additional factor which may play a crucial role in dichlorvos-induced mortality in developing leafcutting bees.

In research to identify possible alternatives to the use of dichlorvos for control of alfalfa leafcutting bee parasites, various pyrethrin-based compounds formulated as liquid sprays were tested for efficacy in control of parasites and prevention of re-parasitism, and for bee safety. Tests were undertaken in bee incubation systems at the field level, but cooperating producers found that the residual effect of the compounds was not adequate to control parasites efficiently.

A second set of pyrethrin-based compounds identified and tested for parasite control and leafcutting bee safety are unique in that they are formulated as pressurized aerosols for use in an automated continuous release system, which may facilitate parasite control with pyrethrin compounds having a relatively short residual period. Research with a pyrethrin aerosol compound designated DS has indicated that treatment in the incubator at 200 mg/hour/100ft^3 is safe for use up to day 11 of bee incubation. Data indicate that after 12 days of DS spray treatment, post-emergent mortality in the bees increases significantly. The average re-parasitism in small-scale tests with DS compound was 4.0% and DS spray was not deleterious to bee emergence or survival in these tests.

Another compound, designated NX93, was also evaluated for efficacy as a parasite control agent and safety to leafcutting bees. In one comprehensive series of tests with three rates of NX93 (1%, 5%, and 10%), re-parasitism averaged 18.0%, 1%, and 0%, respectively. Re-parasitism in untreated controls was 45%. In tests to determine bee safety at the three rates, percent adult emergence and mean time to emergence did not differ significantly from untreated controls. Both DS pyrethrin aerosol spray and NX93 compound appear to be highly efficacious for parasite control and safe to developing and emerging alfalfa leafcutting bees. It is anticipated that data collected from field-scale research on these parasite control alternatives will lead to registration within the next two years.

Research to develop new technology for control of leafcutting bee-related microflora has shown that the fumigant paraformaldehyde is a highly efficaceous compound for control of moulds, yeasts, and bacteria on the surface of leafcutting bee cells. Paraformaldehyde was registered for use in decontamination of bee cells in September, 1993; this registration was based on research undertaken as part of this project; an extension bulletin outlining methods for safe handling and use of paraformaldehyde for bee cell fumigation was published in October, 1993 and distributed to Saskatchewan alfalfa seed producers. Paraformaldehyde fumigation was also been investigated to determine its efficacy for control of chalkbrood. Collaborative research undertaken in 1992 and 1993 with colleagues in southern Alberta, utilizing fumigation technology developed in Saskatchewan as part of this project, has demonstrated that paraformaldehyde fumigation provides significant control of chalkbrood disease.

B. Technical Report

I. Abstract

Surveys of Saskatchewan leafcutting bee populations in 1992 and 1993 identified a significant outbreak of chalkbrood disease (Ascosphaera aggregata) in a population in the northeast part province. Surveys have also shown that levels of the chalcid parasite Pteromalus venustus were lower in many bee populations during 1992 and 1993 due to cool, wet field conditions. These unfavourable conditions during July and August of the past two years have impacted negatively on bee reproduction and have also been conducive to the increased growth of moulds, yeasts, and bacteria associated with the alfalfa leafcutting bee. Research on the chemical dichlorvos, currently used for parasite control, has demonstrated that dichlorvos treatment during spring bee incubation may cause mortality in developing leafcutting bee pupae even at the recommended rate of 0.75g/28.3m^3. This bee mortality is related to dichlorvos rate, treatment time, number of bee cells treated per unit area, position of bee cells in the incubator, and incubation humidity. Alternative compounds have been tested for their potential as parasite control agents during spring incubation and for their safety to developing leafcutting bee pupae. Tests on pyrethrin-based DS compound and on a compound designated NX93 have been undertaken to develop optimum treatment periods and rates. Research has demonstrated that the fumigant paraformaldehyde is a highly efficaceous compound for control of microflora on leafcutting bee cell surfaces and and for control of chalkbrood disease; paraformaldehyde has subsequently been registered for fumigation of alfalfa leafcutting bee cells.

II. Project Background

The alfalfa leafcutting bee (Megachile rotundata) is an important pollinator of alfalfa for seed production and has been used by Saskatchewan alfalfa seed producers as a dependable pollinator for over twenty-five years. At present, over 25,000 ha are devoted to pedigreed alfalfa seed production in Saskatchewan, with another 10,000 ha in common seed production. Sales of alfalfa seed earn producers up to $12 million annually, while sales of surplus leafcutting bee cells bring Saskatchewan alfalfa seed producers an additional $4 - $6 million annually. New grower interest is strong as producers seek to diversify their agricultural operations.

Infestations of the chalcid parasite, Pteromalus venustus, are a major problem in western Canadian populations of the alfalfa leafcutting bee. The parasite larvae in a parasitized leafcutting bee cell develop to the adult stage so rapidly that multiple generations may occur in a relatively short period of time. This creates the potential for a large build-up in parasite levels within a leafcutting bee population in a single season.

Alfalfa seed producers currently use the chemical dichlorvos (2, 2 - dichlorovinyl dimethyl phosphate) in the resin strip formulation known as Vapona^{R} for control of parasites during spring bee incubation. A trend to increased parasitism in leafcutting bee populations has resulted in many producers using dichlorvos for spring parasite control at rates which exceed the recommended rate of 75g/28.3m^3, and using dichlorvos for extended time periods.

Recent research has indicated that significant increases in leafcutting bee mortality during incubation can be related to dichlorvos rate, treatment time, and number of bee cells treated. Studies by the U.S. Environmental Protection Agency have demonstrated that dichlorvos is a carcinogen and is also implicated in liver and nervous system damage. There is thus a strong possibility that production of dichlorvos resin strips may be halted in the near future. To date, there is no alternative to the use of dichlorvos for parasite control in alfalfa leafcutting bee populations.

Another major problem affecting leafcutting bee populations is chalkbrood disease, caused by the fungus Ascosphaera aggregata. Chalkbrood disease affects leafcutting bee larvae after they ingest spores of the fungus while feeding on pollen. Once the larvae die, millions of spores may form on the surface of the cadaver. Chalkbrood is currently the most serious limiting factor to leafcutting bee populations and alfalfa seed production in the northwestern U.S. and as a result, the U.S. has become the major market for Canadian leafcutting bee cells. Chalkbrood disease has become a chronic problem in southern Alberta and occurs sporadically in Manitoba and Saskatchewan as well.

A number of other fungal species are also found in association with alfalfa leafcutting bee populations. These moulds contaminate adult leafcutting bees, bee cells, and nest material, and have been implicated in human allergic reactions and respiratory diseases. Recent research to develop new technology for control of moulds, yeasts, and bacteria in leafcutting bees resulted in the discovery of paraformaldehyde fumigation as an efficacious method for control of leafcutting bee-related microflora.

In order to determine the status of parasites, chalkbrood disease, and moulds in Saskatchewan leafcutting bee populations, to examine current methods of parasite control for bee safety, and to develop new technology to control parasite and chalkbrood problems, a research project was undertaken with the following objectives:

1. To determine the levels of chalcid parasitism (Pteromalus venustus), chalkbrood disease (Ascosphaera aggregata), and moulds in Saskatchewan alfalfa leafcutting bee populations by surveying bee samples from alfalfa seed producers and by nest-trapping leafcutting bees at sites throughout the province.

2. To evaluate current methods for control of chalcid parasites in alfalfa leafcutting bee populations and examine the effects of these methods on the viability of leafcutting bees.

3. To identify and test alternative compounds for control of chalcid parasites in alfalfa leafcutting bee populations and to transfer alternative parasite control technology to alfalfa seed producers.

4. To develop and transfer to producers new technology for decontaminating the surfaces of leafcutting bee cells and for controlling chalkbrood disease and thus enhance the quality of alfalfa leafcutting bee populations.

III. Project Activities / Results / Conclusions

1. Saskatchewan alfalfa leafcutting bee survey

a) 1992/93 and 1993/94 winter surveys

In order to survey Saskatchewan leafcutting bee populations for levels of chalkbrood disease and chalcid parasites, samples of bee cells were solicited annually from producers throughout the province. Samples were stored at 4°C upon receipt. From each sample, 4 x 15 g subsamples were arranged in polystyrene nest boards and x-rayed with an H.P. Faxatron Unit (14kvp @ 10 sec) using Kodak X-Omat AR film. Exposed x-ray films were then evaluated using a Picker FVS illuminator. Cells not containing apparently healthy bee prepupae were noted on the x-ray, dissected, and their contents analysed and tabulated. Following evaluation of samples, data collected were returned to producers. The data summary presented in Table I is an overview of results from the 1992/93 Saskatchewan leafcutting bee survey.

Table I. 1992/93 Saskatchewan Leafcutting Bee Survey Data.

CATEGORY	STATISTIC (%) Mean	sd.	High	Low
HEALTHY PREPUPAE	73.1	9.6	88.8	48.1
DEAD LARVAE/PREPUPAE				
Discoloured	4.3	1.9	10.3	0.8
Mouldy	0.19	0.25	1.22	0.00
Ascosphaera aggregata	0.017	0.14	1.10	0.00
Non-spor.Chalkbrood	0.031	0.18	1.40	0.00
Other Ascosphaera spp.	0.020	0.06	0.30	0.00
MOULDY COCOONS				
Pollen Mould	3.19	3.72	23.65	0.00
Pollen Ascosphaera spp.	1.45	2.05	10.52	0.00
PARASITES	0.37	1.34	10.66	0.00
SECOND GENERATION	0.3	0.4	1.8	0.0
POLLEN BALLS	13.0	6.6	35.0	2.9
CRUSHED COCOONS	3.7	5.6	30.5	0.0
OTHER	0.3	0.6	3.8	0.0
LIVE COUNT/LB.	3859	525	4989	2460
LIVE COUNT/KG.	8495	1159	10986	5418

The 1992/93 Saskatchewan leafcutting bee survey, extended to mid-May, 1992, involved analysis of 84 samples from the populations of 64 Saskatchewan producers. A significant chalkbrood infestation was identified in a single leafcutting bee population from cell samples received in late April, 1993. In this population, located in the northeast part of the province, the level of chalkbrood discovered was 1.40% sporulating and 1.07% non-sporulating. Occurrence of dead larvae infected with other Ascosphaera species was also higher than the survey average (0.30% in the chalkbrood-infested population). This is the first significant outbreak of chalkbrood to be identified in a Saskatchewan alfalfa leafcutting bee population. Evaluation of other bee populations in this area of the province indicated no evidence of additional chalkbrood infestations.

Following identification of chalkbrood in this leafcutting bee population, the producer involved was immediately advised on procedures to prevent the spread of the disease and to assist in its eradication. All nest material and bee cells from the infested population were fumigated with paraformaldehyde prior to the 1993 field season, utilizing protocols from technology developed through research undertaken as part of this project. As well, field shelters were sprayed with a bleach solution as part of the clean-up procedure.

While no other incidence of sporulating chalkbrood was noted in the province in the 1992/93 survey, nonsporulating chalkbrood was observed at two other locations in widely separated leafcutting bee populations. Occurrence of other Ascosphaera species was low overall in the larval category (0.020%), but moderately higher in the pollen Ascosphaera spp. category (1.45%). The category entitled "other Ascosphaera spp." refers to native Ascosphaera mould species which are not classic chalkbrood (A. aggregata), but which do occur at low levels in some populations and which may be pathogenic to leafcutting bee larvae. The category entitled "pollen Ascosphaera spp." refers to native Ascosphaera mould species found growing on pollen balls.

A brief overview of other data collected in the 1992/93 leafcutting bee survey indicated that the healthy prepupae average, at 73.1%, was down significantly when compared with data over the previous three year period (Appendix A). This decrease in healthy prepupae average was attributed to a corresponding significant increase in the average pollen ball level, which at 13.0% was at its highest point in the five year period. This increase in pollen ball levels was largely due to the cool, wet conditions occurring during July and August, 1992, which lowered leafcutting bee productivity through a decrease in flying time and an increase in egg and early instar larval mortality. Larval and pollen moulds, at 0.19% and 3.19% respectively, remained at levels comparable with previous years. Other categories, including discoloured larvae, pollen Ascosphaera spp., second generation, and crushed cocoons, remained at levels similar to previous survey results.

Dead discoloured larvae showed a slight upward trend compared with previous levels, likely due to summer weather conditions. Parasite counts, at 0.37%, were lower than in previous years and once again this trend is due to the poor weather conditions experienced during the field season. In a year that is poor for their hosts in terms of production of bee larvae, the chalcid parasite will also experience reproduction problems.

The 1993/94 leafcutting bee survey was completed in late March, 1994, with a total of 97 samples from the bee populations of 74 Saskatchewan producers received and analysed. An examination of 1993/94 statistical data, which are given in Table II, indicates that parasite levels ranged as high as 2.15%, with the average parasite count at a level of 0.14%. The only incidence of chalkbrood disease noted in the 1993/94 survey was from the population previously identified in the 1992/93 survey. In this population, located in the northeast of the province, the level of chalkbrood was 0.54% sporulating and 0.32% nonsporulating. The level of disease in both categories was down substantially from the previous year. However, occurrence of other larval Ascosphaera species in this population was found at a very high level (7.86%).

Table II. 1993/94 Saskatchewan Leafcutting Bee Survey Data.

CATEGORY	STATISTIC (%)			
	Mean	sd.	High	Low
HEALTHY PREPUPAE	70.6	11.1	89.1	42.8
DEAD LARVAE/PREPUPAE				
Discoloured	5.6	2.9	13.8	1.5
Mouldy	0.29	0.42	2.35	0.00
Ascosphaera aggregata	0.007	0.06	0.54	0.00
Non-spor.Chalkbrood	0.004	0.04	0.32	0.00
Other Ascosphaera spp.	0.128	0.90	7.86	0.00
MOULDY COCOONS				
Pollen Mould	6.62	5.27	22.85	0.14
Pollen Ascosphaera spp.	2.54	4.53	26.40	0.00
PARASITES	0.14	0.35	2.15	0.00
SECOND GENERATION	0.2	0.4	2.3	0.0
POLLEN BALLS	10.9	5.4	28.4	2.3
CRUSHED COCOONS	2.6	2.3	10.3	0.0
OTHER	0.3	0.4	1.5	0.0
LIVE COUNT/LB.	4069	536	5420	2831
LIVE COUNT/KG.	8966	1186	11936	6235

Despite this one unusual occurrence, the level of other Ascosphaera species on larvae continued at a relatively low level overall in the survey (0.128%), while pollen Ascosphaera species were seen in 2.54% of cells analysed. Larval and pollen moulds, at 0.3% and 6.6% respectively, were at or above levels observed during 1989-1992 (Appendix A). As in the 1992/93 survey, an increase in dead discoloured larvae and high pollen ball levels are due to the cool, wet conditions which occurred once again in July and August, 1993. These conditions are also responsible for the increase in all categories of larval and pollen-related moulds.

In order to evaluate the status of parasites in Saskatchewan leafcutting bee populations over the past nine years, data collected in previous surveys were analysed and summarized. Chalcid parasite (Pteromalus venustus) levels from 1985/86 through 1993/94 are presented in Table III. As shown in the table, chalcid parasites were found at lower levels in 1992/93 and 1993/94 surveys. As noted previously, leafcutting bee populations experience severe problems with reproduction in cool, wet years such as 1992 and 1993, and chalcid parasites which rely on bee larvae to reparasitize are also adversely affected by these conditions.

Table III. Chalcid parasite levels in surveyed Saskatchewan leafcutting bee populations.

YEAR	STATISTIC (%)			
	Mean	S.Dev.	High	Low
1985/86	0.52	1.16	6.5	0.0
1986/87	1.50	6.90	44.9	0.0
1987/88	0.50	1.06	5.7	0.0
1988/89	2.10	3.40	20.7	0.0
1989/90	1.77	2.86	12.7	0.0
1990/91	1.06	1.55	7.3	0.0
1991/92	1.82	3.43	18.0	0.0
1992/93	0.38	1.38	10.7	0.0
1993/94	0.14	0.35	2.2	0.0

Parasitism by species other than chalcid parasites was also examined in the 1992/93 and 1993/94 leafcutting bee surveys. Coelioxys spp. are parasites which belong to the same family as the alfalfa leafcutting bee.

In the 1992/93 survey, these parasites were present in 23.3% of populations surveyed, and they occurred at levels of up to 0.44%. In the 1993/94 survey, Coelioxys spp. were present in 18.2% of populations, occurring at levels of up to 0.46%. Data indicate that Coelioxys spp. parasites are present in 16.7% to 33.3% of leafcutting bee populations in a given year but occur at much lower levels than chalcid parasites. In many alfalfa seed producing areas of Europe, Coelioxys spp. parasitism is the most severe problem threatening leafcutting bee populations. Since the potential for this parasite to become a serious pest does exist, monitoring of Coelioxys spp. parasites continues as part of the Saskatchewan leafcutting bee survey.

An overview of levels of sporulating chalkbrood , non-sporulating chalkbrood, and other Ascosphaera species found in Saskatchewan alfalfa leafcutting bee surveys during the period 1985/86 through 1993/94 is given in Table IV. Sporulating A. aggregata is the infectious form of chalkbrood disease, where spores are produced on infected cadavers and spread throughout a population on the bodies of other bees. Non-sporulating chalkbrood involves a process of the disease where no spores are formed, and thus the disease in this form is not infectious. The category entitled "other Ascosphaera spp." refers to native Ascosphaera species (i.e. A. larvis, A. pollenicola, and A. variegata) which are not classic chalkbrood (A. aggregata), but which do occur in some populations and which may be pathogenic to leafcutting bee larvae.

Table IV. Levels of Ascosphaera in Saskatchewan alfalfa leafcutting bee surveys 1985/86 - 1993/94.

YEAR	NO. SAMPLES EVALUATED	A. aggregata SPORULATING (%)	A. aggregata NON-SPOR. (%)	OTHER Asco. SPECIES (%)
1985/86	60	0.0	0.002	0.019
1986/87	42	0.0	0.0	0.0
1987/88	59	0.0	0.0	0.045
1988/89	71	0.002	0.002	0.002
1989/90	76	0.0	0.009	0.007
1990/91	55	0.0	0.0	0.011
1991/92	61	0.0	0.003	0.005
1992/93	84	0.017	0.031	0.020
1993/94	97	0.007	0.004	0.128

From this table, it can be observed that chalkbrood has occurred only sporadically and at very low levels in Saskatchewan over the survey period. Since this most recent and notable outbreak in northeast Saskatchewan is currently confined to a single population, it may be possible to totally eradicate the disease at that location. In the 1993/94 alfalfa leafcutting bee survey an increase was noted in the number of bee samples submitted, particularly from alfalfa seed producers in the northeast part of the province.

b) 1992 and 1993 trapnest project

To supplement data collected in the annual Saskatchewan leafcutting bee / chalkbrood survey undertaken during each winter, monitoring of chalkbrood disease in domestic and native leafcutting bee populations is undertaken during the summer by placement of specially-constructed trapnests at sites in close proximity to alfalfa seed fields throughout the province. Analysis of trapnest-collected material helps to provide an early warning should an infestation of chalkbrood disease, severe chalcid parasitism, or some other problem occur in a particular area of the province.

During the 1992 field season, a total of 25 trapnests were placed in alfalfa leafcutting bee shelters and in sites adjacent to alfalfa seed fields at 13 locations in Saskatchewan (Appendix B). Prior to deployment, wood nest boxes were sterilised and then filled with new polystyrene laminate nest material to provide 270 nest tunnels in each trapnest. The trapnests remained in the field from mid-June to mid-September; cells were harvested and representative cell samples from each trapnest were analysed in early October in order to obtain information on domestic and native leafcutting bee species, associated moulds, and parasites.

Analysis of representative cell samples, comprising 6127 of the domestic leafcutting bee cells (Megachile rotundata) collected in the 1992 trapnests (Table IV), indicated that no cadavers infected with chalkbrood caused by Ascosphaera aggregata were present. Analysis of a total of 331 native leafcutting bee cells (Megachile relativa) collected in 1992 trapnests (Table IV) indicated that there was no chalkbrood present in the native bee cells evaluated. Past observations of chalkbrood in native leafcutting bee species in Saskatchewan indicate that the native species may provide a pathway for movement of A. aggregata into domesticated populations of M. rotundata; for this reason, native bee species are monitored as part of the trapnest survey.

The samples analysed from trap nests are not broken and tumbled as they would be in a commercial operation and the healthy prepupal count given in Table IV is thus lower than might be expected in producer bee samples. Analysis is carried out in this way in order to efficiently monitor any disease, parasites, or predators which may be present in the population.

Data for M. rotundata indicate that pollen balls, pollen mould, and pollen Ascosphaera spp. were found at very high levels in some 1992 trapnest samples.

Table V. 1992 Saskatchewan trapnest project data from analysis of representative cell samples.

CATEGORY	STATISTIC (%)			
	M. rotundata		M. relativa	
	No.	%	No.	%
HEALTHY PREPUPAE	4173	68.1	274	82.8
DEAD LARVAE/PREPUPAE				
Discoloured	287	4.7	16	4.8
Mouldy	26	0.4	4	1.2
Ascosphaera aggregata	0	0.0	0	0.0
Non-spor.Chalkbrood	0	0.0	0	0.0
Other Ascosphaera spp.	0	0.0	0	0.0
MOULDY COCOONS				
Pollen Mould	427	7.0	0	0.0
Pollen Ascosphaera spp.	277	4.5	1	0.3
PARASITES	25	0.4	6	1.8
SECOND GENERATION	15	0.2	0	0.0
POLLEN BALLS	882	14.4	27	8.2
OTHER	15	0.2[a]	3	0.9[a]
NO. COCOONS ANALYSED	6127		331	

[a] Includes cells in crushed/damaged category and cells with contents depredated by stored products pests (e.g. beetles).

During the 1993 field season, a total of 27 trapnests were placed in alfalfa leafcutting bee shelters and in sites adjacent to alfalfa seed fields at 14 locations in Saskatchewan. Analysis of representative cell samples, comprising 6149 of the domestic leafcutting bee cells (Megachile rotundata) collected in the 1993 trapnests (Table VI), indicated that cadavers infected with chalkbrood caused by Ascosphaera aggregata were present at two trapnest locations. At one location, 3 sporulating and 3 non-sporulating chalkbrood cadavers were identified, while at a second location, 1 non-sporulating cadaver was identified. The first location, from which both sporulating and non-sporulating chalkbrood cadavers were isolated, was at the site of the chalkbrood outbreak previously reported from northeastern Saskatchewan.

Analysis of a total of 186 native leafcutting bee cells (Megachile relativa) collected in five of the 1993 trapnests indicated that there was no chalkbrood present in the native bee cells evaluated. An additional 56 wood block trapnests were placed at 26 sites as part of a joint Alberta/Saskatchewan/Manitoba project to determine the incidence of chalkbrood in native leafcutting bee species on the prairies. This ongoing project to identify chalkbrood in native leafcutting bee species will help to protect domesticated populations of M. rotundata from the disease.

Table VI. 1993 Saskatchewan trapnest project data from analysis of representative cell samples.

CATEGORY	STATISTIC (%)			
	M. rotundata		M. relativa	
	No.	%	No.	%
HEALTHY PREPUPAE	4342	70.6	157	84.4
DEAD LARVAE/PREPUPAE				
Discoloured	296	4.8	5	2.7
Mouldy	44	0.7	0	0.0
Ascosphaera aggregata	3	0.05	0	0.0
Non-spor.Chalkbrood	4	0.07	0	0.0
Other Ascosphaera spp.	107	1.74	1	0.5
MOULDY COCOONS				
Pollen Mould	592	9.6	7	3.8
Pollen Ascosphaera spp.	316	5.1	0	0.0
PARASITES	30	0.5	6	3.2
SECOND GENERATION	2	0.03	0	0.0
POLLEN BALLS	409	6.7	5	2.7
OTHER	4	0.07[a]	5	2.7[b]
NO. COCOONS ANALYSED	6149		186	

[a] Includes cells with contents depredated by stored products pests (e.g. beetle larvae, Trogoderma sp.).
[b] Includes cells parasitized by Coelioxys sp.

In cases where a chalkbrood cadaver is identified in the Saskatchewan trapnest survey or in the Saskatchewan leafcutting bee survey, a policy of producer confidentiality is maintained, and producers involved are advised on use of technology to decontaminate nest material and bee cells; as well, trapnest sampling and chalkbrood surveys are undertaken at these locations in subsequent years.

2. Leafcutting bee parasite control research

a) Evaluation of current parasite control

Alfalfa seed producers currently use the chemical dichlorvos (2, 2 - dichlorovinyl dimethyl phosphate) in the resin strip formulation known as Vapona^{R} for control of parasites during spring bee incubation. A trend to increased parasitism in alfalfa leafcutting bee populations has resulted in many producers using dichlorvos for spring parasite control at rates which exceed the recommended rate of 75g/28.3m^3 (0.75 resin strip/1000 ft^3) and also using dichlorvos treatment early in the incubation cycle rather than restricting its use to the 7-14 day period as recommended. Research undertaken previously has indicated that leafcutting bee mortality increases with increasing dichlorvos rates and early treatment. As well, recent studies by the U.S. Environmental Protection Agency, have demonstrated that dichlorvos is a carcinogen and is also implicated in liver and nervous system damage. There is thus a possibility that production of dichlorvos resin strips may be halted in the future. To date, there is no alternative to the use of dichlorvos for parasite control in alfalfa leafcutting bee populations.

Dichlorvos-induced mortality in developing leafcutting bees was examined previously (1990-1992) in relation to dichlorvos rate, treatment time, number of bees treated, and position of bees in the incubation tray. Dichlorvos rate is directly related to bee mortality; at 1x the recommended rate, bee emergence in the incubator averages 70.8%, while at 4x the recommended rate, emergence is reduced to 24.0%. Treatment time is also an important factor; treatment at 1x the recommended rate during day 7-14 of the incubation period will result in bee emergence of >70%, while use of the same rate during day 0-14 of incubation results in emergence of <55%. Since dichlorvos treatment is undertaken based on the volume of the incubator (i.e. 0.75 resin strip/1000 ft^3) the number of leafcutting bees placed in the incubator is also critical. In treatments of 400 and 4000 bee cells/ft^3 at the 1x dichlorvos rate during day 7-14 of incubation, emergence in the 4000 cell treatment was 70.8%, while in the 400 cell treatment, emergence was 4.2%. In work to evaluate bee mortality related to bee cell position in the incubation tray, a comparison of emergence from cells on the surface of trays and cells randomly selected from throughout the depth of the trays indicated that emergence from cells in the top layer (32.0%) was significantly lower than emergence from randomly selected cells (70.8%).

Dissection of unemerged bee cells indicates that a teratogenic effect (i.e. one causing abnormal pupal development) has occurred. The characteristic teratogenic phenotype is a late-stage pupae or pre-emergent adult with poorly differentiated head, thorax, and abdomen, little or no apparent development of appendages, and retarded wing development. This phenotype was observed in all experiments and was more apparent with increasing dichlorvos rate.

The most recent research undertaken on dichlorvos-induced mortality in developing alfalfa leafcutting bees in the incubator has involved an examination of dichlorvos and relative humidity. This work utilized treatment of bees (1000 bee cells/ft^3) at the 1x dichlorvos rate during day 7-14 of incubation under low (28%), ambient (59%), and high (84%) humidity conditions. An emergence profile of control and treated cells is given in Table VII. In the control cell treatment, incubation was undertaken at the ambient humidity (59%).

Table VII. Percent emergence and emergence time (days) in dichlorvos-treated bee cells at various humidities.

TREATMENT	EMERGENCE (%)	TIME TO EMERGENCE (DAYS)		
		male	female	combined
Control	72.0%	20.25	21.58	20.69
Low RH (28%)	27.0%	19.44	20.55	19.89
Amb. RH (59%)	28.0%	19.94	20.42	20.14
High RH (84%)	79.0%	19.66	22.22	20.41

Cumulative emergence data are presented in Appendix C. The effect of relative humidity in the incubator on dichlorvos-induced bee mortality is significant in the low to moderate humidities examined in this study. Adult emergence from cells treated with dichlorvos at the recommended rate from day 7-14 was very low at humidities of 28% and 59%. Dichlorvos-induced mortality in developing leafcutting bee pupae is apparently increased with lower humidities due to increased dichlorvos release in a low humidity atmosphere and due to increased dichlorvos uptake by the developing bee pupa across the bee cell surface. Alfalfa seed producers incubate leafcutting bees at various relative humidities, and incubation humidity is often affected by weather conditions (i.e. wet or dry/warm or cool spring/summer). As noted above, dichlorvos rate, treatment time, number of bees treated, and position of bees in the incubation tray may all significantly affect bee emergence. Relative humidity is an additional factor which may play a crucial role in dichlorvos-induced mortality in developing leafcutting bees.

b) Evaluation of alternative compounds for parasite control

Research to identify possible alternatives to dichlorvos for control of chalcid parasites has involved work in the laboratory followed by field-scale experiments. Laboratory research to test candidate compounds for their potential as parasite control agents has involved work in the following areas... treatment of parasitized cells to determine efficacy for control of newly emerged parasites, release of mated female parasites on treated cells containing healthy bee prepupae to determine rate of re-parasitism, and treatment of healthy bee cells to determine the safety of the compounds to developing bee prepupae and emergent adult bees.

Research to assess pyrethrin aerosol and pyrethrin liquid spray compounds for their relative efficacy in control of newly emerged Pteromalus venustus parasites has involved treating groups of parasitized cells with DS and PY9 aerosol sprays or with TRO, INS, and PBO liquid sprays. All spray treatments were carried out immediately prior to incubation at 30°C/40%R.H.

During the parasite emergence period (ca Day 6-10) parasite mortality in each of the treatments was assessed at 24 hour intervals. The graph presented in Appendix D indicates that DS aerosol spray provided the highest initial rate of control at Day 06 (71.6%, compared with 16.1% for untreated control - CON). TRO compound was the most efficient of the pyrethrin liquid sprays, with control of 40.1% at Day 06.

Laboratory research was continued with pyrethrin liquid sprays designated TRO and PBO. These sprays are formulated with lower amounts of pyrethrins than the aerosol compounds and break down rapidly following application. When mated female chalcid parasites were released on healthy bee cells sprayed with these compounds, parasite mortality averaged 76.9% and 60.0%, respectively, for TRO and PBO within 24 hours. Re-parasitism of bee cells averaged 10.0% in TRO treatments, 5.0% in PBO treatments, and 50.0% in untreated controls. Percent adult emergence from control and treated cells was not significantly different.

In a field test of TRO compound, an air pressurized unit was used to spray TRO over cells in incubation trays at a rate of 4.5 g active ingredient per day from day 7 (onset of parasite emergence) through day 9. However, as parasite emergence peaked on day 9/10 of incubation, the high numbers of chalcid parasites could not be adequately controlled by TRO treatment. It was found that while TRO was effective as a contact spray for parasite control, the residual effect was too short to be efficacious in this test. The low residual toxicity of TRO to emerging adult bees may make it a useful contact spray for spot parasite control use (i.e. direct spraying of cells in trays with high parasite emergence).

Further laboratory work with pyrethrin aerosol compounds DS and PY9 showed that when mated female chalcid parasites were released on healthy leafcutting bee cells sprayed with these compounds, parasite mortality averaged 97.5% and 72.5%, respectively, for DS and PY9 within 24 hours. Re-parasitism of bee cells was 0% in both treatments and 100% in untreated control cells. Since subsequent incubation tests of the treated bee cells indicated a level of mortality due to poisoning in emerging adult bees, a second series of tests was undertaken in which healthy bee cells were treated with 50 mg or 150 mg active ingredient DS/PY9 per replicate at day 0, day 7, or day 14 of the incubation period. Results of these tests indicated that while there were no significant differences in percent adult emergence between untreated controls and 50 mg DS/PY9 treatments, emergence was significantly lower from cell replicates treated with 150 mg, especially in PY9 treatments. Post-emergent mortality due to adult bee poisoning was observed in all treatments but was highest in PY9 tests.

DS compound, which contains a lower concentration of pyrethrins and piperonyl butoxide than PY9 compound, was selected for the first pyrethrin aerosol spray field-scale test. In this test, 1.3 million bee cells from populations with parasitism ranging from 4-9% were placed in trays (50,000 cells per tray) and either pre-treated with sprays of 500 mg or 1000 mg active ingredient DS applied directly on the cells in each tray or left without pre-treatment. Following pre-treatment at day 4 of the incubation period, one automated aerosol dispenser was turned on in the incubator. At the onset of parasite emergence on day 7, additional direct spray treatments of cells in trays were carried out. However, parasites emerging from cells in trays not pre-treated with DS on day 4 of incubation were not adequately controlled by DS from these later direct spray treatments or by DS from the automated dispenser. Following collection of cell samples from all trays, DS treatment was terminated and dichlorvos was then used during the remainder of the parasite control period.

A second test of DS compound involved incubation of 250,000 bee cells with parasitism over 4%. Bee cells in this test were either left without pre-treatment spray or pre-treated with direct sprays of 500 mg, 1000 mg, or 1500 mg active ingredient DS per tray; four automated aerosol dispensers were then utilized from Day 04 through Day 14 of the incubation period. The level of parasite control observed was equivalent to that achieved with dichlorvos under ideal conditions. Large numbers of male and female parasites were dead in trays and on the floor of the incubator and few female parasites moving about in the trays. Groups of cells removed from the trays during this period were incubated in the laboratory to determine re-parasitism, adult bee emergence, and extent of post-emergent bee poisoning. Overall re-parasitism was determined to be approximately 0.4%. Percent adult emergence and post-emergent survival of adults from leafcutting bee cells receiving various treatments with DS pyrethrin aerosol spray are given in Table VIII.

Table VIII. Percent adult emergence and post-emergent survival of adults from leafcutting bee cells treated with DS compound for parasite control during spring incubation.

TREATMENT[a]	PERCENT EMERGENCE	MEAN TIME TO EMERGENCE (days)	POST-EMERGENT SURVIVAL (%)
0 mg	96.9	25.35	91.2
500 mg	95.7	25.52	31.7
1000 mg	97.1	25.59	13.0
1500 mg	95.2	25.98	0.0

[a] All treatments comprised indirect spray via automated aerosol dispenser at a rate of 800 mg/hour in addition to pre-treatment direct spray specified above (i.e. 0, 500, 1000, or 1500 mg DS).

While percent emergence did not differ significantly among treatments, post-emergent survival decreased dramatically with increasing pre-treatment direct spray rate, indicating that the residual effect of spray treatments applied directly on cells was more lethal to emerging adult bees than the residue from indirect background spray generated at regular intervals by automated aerosol dispensers. Continuing work to identify optimum treatment rate and period of treatment, and reduce the occurrence of post-emergent adult bee mortality, was therefore undertaken through a comprehensive experiment which involved spray treatment of replicates of healthy leafcutting bee cells with the compound DS over a series of treatment times.

The data collected from this experiment indicated that treatment at a rate of ca 200 mg DS/hour/100ft^3 may be safe to emerging adult leafcutting bees if not continued past day 11 of bee incubation (Table IX). Data indicate that after 12 days of DS spray treatment, post-emergent mortality in the bees increases significantly to 19.4%, although percent emergence is unaffected.

To further evaluate the parasite control potential of the pyrethrin aerosol spray system, untreated control and DS experiments were run consecutively in a small research incubator to determine the level of re-parasitism of healthy bee cells by the chalcid parasite Pteromalus venustus.

Table IX. Post-emergent mortality in adult M. rotundata emerging from leafcutting bee cells treated with DS compound over a series of time periods during incubation.

TREATMENT	PERCENT EMERGENCE	MEAN TIME TO EMERGENCE (days)	POST-EMERGENT MORTALITY (%)
Control	66.7	23.05	0.8
0 - 1	63.5	23.09	0.8
0 - 2	65.8	22.92	0.8
0 - 3	62.7	22.80	0.0
0 - 4	68.7	23.26	0.0
0 - 5	64.2	23.06	0.0
0 - 6	72.7	22.38	2.0
0 - 7	56.9	22.62	1.8
0 - 8	62.9	23.37	1.6
0 - 9	63.0	22.51	4.0
0 - 10	70.3	22.85	8.0
0 - 11	64.0	22.90	6.1
0 - 12	65.5	23.20	19.4
0 - 13	63.7	22.69	13.3
0 - 14	66.0	22.58	25.4
0 - 21	66.0	23.37	46.3

In each of the tests, ten replicates of 100 cells containing healthy bee prepupae were incubated at 30°C/60%RH with 55-80 parasitized cells. In the control test, the only parasite control mechanism in place was a passive UV light/water bath system. In the pyrethrin spray test, cells were treated with 200 mg DS/hour/100ft^3 from day 0 to day 11 of incubation; the UV light/water bath system was also in place.

Results of the control and DS tests are given in Table X, based on dissection of 50 cells per replicate in each of the two tests (n=500). Re-parasitism of healthy cells averaged 39.6% (range 6.0-72.0%) in the control test. Re-parasitism in the SM-DS spray test averaged 4.0% (range 0.0-18.0%); mean percent reduction in the level of re-parasitism was 92.4% (range 71.0-100.0%).

Safety of DS spray to developing bee pupae and emerging adult bees was also evaluated in small-scale tests. Data on percent emergence and post-emergent survival are given in Table XI.

Table X. Effect of DS/pyrethrin aerosol spray treatment on control of re-parasitism by P. venustus.

REPLICATE NO.	RE-PARASITISM (%) CONTROL	DS TREATED	% DECREASE IN RE-PARASITISM
01	6.0	0.0	100.0
02	12.0	0.0	100.0
03	30.0	2.0	93.3
04	40.0	4.0	90.0
05	22.0	0.0	100.0
06	48.0	6.0	87.5
07	50.0	4.0	92.0
08	54.0	4.0	92.6
09	62.0	18.0	71.0
10	72.0	2.0	97.2
Mean	39.6	4.0	92.4
sd.	21.7	5.3	8.7

These data indicate that DS spray treatment at this scale was not deleterious to bee emergence (control 92.5%, treated 94.9%); data on post-emergent survival (control 98.4%, treated 93.9%) indicate a slight decrease in survival among adults emerging from treated cells, mainly from mortality in early emerging male bees. This mortality may be due to incomplete breakdown of pyrethrin residues at the time of initial bee emergence. A system of feeding newly-emerged adult bees has been devised in order to examine post-emergent survival in more detail; this system will be incorporated in future experiments.

Table XI. Effect of DS/pyrethrin aerosol spray treatment on post-emergent survival of M. rotundata.

REPLICATE NO.	EMERGENCE (%)	DEAD ADULTS male: female	POST-EMERGENT SURVIVAL (%)
CONTROL[a]			
01	92.0	1 : 0	98.9
02	89.0	3 : 0	96.6
03	92.0	-	100.0
04	97.0	1 : 1	97.9
Mean	92.5	1.25 : 0.25[c]	98.4
sd.	3.3	-	1.4
DS TREAT.[b]			
01	95.0	11 : 0	88.4
02	94.9	5 : 0	94.6
03	95.0	4 : 0	95.8
04	94.7	3 : 0	96.6
Mean	94.9	5.75 : 0.0[c]	93.9
sd.	0.1	_	3.7

[a] n=100 cells containing healthy prepupae.

[b] n=94-100 cells containing healthy prepupae.

[c] mean no. dead adult males and females in 100 emerging leafcutting bees (i.e. control = 1.6%, DS treat. = 6.1% post-emergent mortality).

Two other compounds have also been investigated for potential as parasite control agents. The first of these compounds, designated NX93, was initially tested on bee cells for its safety to developing and emerging leafcutting bees. Two rates of NX93 (10% and 50%) were evaluated with treatments at day 5, 6, or 7 of incubation. Results indicated that the 50% rate of NX93 caused unacceptable pupal mortality (emergence range 34.7-43.9%) and post-emergent mortality (survival range 34.2-55.8%) at all treatment times; however, the 10% rate of NX93 was much less harmful to developing and emerging bees and the treatment had no significant effect on mean time to emergence when compared with untreated controls.

Work on NX93(10%) was repeated using a pre-incubation treatment (i.e. treatment at day 0 only). A second compound, designated CZ93, was also included in this test, which evaluated the effect of these compounds on bee emergence and on P. venustus re-parasitism.

Table XII. Re-parasitism by P. venustus of control untreated, NX93 treated, and CZ93 treated leafcutting bee cells.

TEST/REPLICATE NO.		RE-PARASITISM (%) Control	NX93(10%)	CZ93(10%)
I	01	16.0	0.0	68.0
	02	40.0	0.0	20.0
	03	56.0	0.0	60.0
	04	60.0	0.0	60.0
	Mean	43.0	0.0	52.0
	sd.	19.96	-	21.67
II	01	62.0	0.0	60.0

In part I of the test (Table XII), healthy adult parasites were given a choice of 25 control, NX93-treated, or CZ93-treated bee cells. In part II of the test, healthy parasites were placed on 50 cells of only one of the three groups (control, NX93, or CZ93). Data from the two parts of the experiment show that NX93(10%) reduced re-parasitism to 0.0%, while re-parasitism was found at levels of 43.0-62.0% in controls and 52.0-60.0% in CZ93(10%) treatments. Adult emergence from control and treated cells not subjected to re-parasitism tests was also determined. Emergence from cells treated with NX93(10%) averaged 98.0% and did not differ significantly from control emergence (92.5%). Emergence from cells treated with CZ93(10%) was significantly lower at 62.0%, indicating that CZ93 compound was deleterious to pupal development in leafcutting bees.

A small-scale experiment to evaluate the effect of various rates of NX93 on re-parasitism was subsequently undertaken; data from this work are given in Table XIII. In part I of this test, healthy adult parasites were given a choice of control, NX93(1%)-treated, or NX93(5%)-treated bee cells; re-parasitism in the three treatments was 45.0%, 18.0%, and 1.0%, respectively.

In part II of the test, parasites were placed on either control, NX93(1%)-treated, NX93(5%)-treated, or NX93(10%)-treated leafcutting bee cells. Re-parasitism levels were similar to those found in part I of the test, with the NX93(10%) treatment once again totally preventing re-parasitism by P. venustus.

Table XIII. Re-parasitism by P. venustus of control untreated and NX93 treated leafcutting bee cells[a]

REPLICATE NO.		Control	RE-PARASITISM (%) NX93-1%	NX93-5%	NX93-10%
I[b]	01	52.0	8.0	4.0	-
	02	48.0	8.0	0.0	-
	03	44.0	36.0	0.0	-
	04	36.0	20.0	0.0	-
Mean		43.0	18.0	1.0	-
sd.		19.96	13.27	2.00	-
II[c]	01	38.0	22.0	4.0	0.0

[a] test I involved 10-14 female PV / replicate.
test II involved 10 female PV / replicate.
treatment at day 0 of incubation only.

[b] n=25 cells containing healthy prepupae per replicate in each treatment.

[c] n=50 cells containing healthy prepupae per replicate in each treatment.

Upon completion of the experiment, additional analysis was undertaken to determine percent adult emergence and mean time to emergence for adult bees in all treatments. This data is presented in Table XIV. It indicates that treatment of leafcutting bee cells with 1%, 5%, and 10% NX93 compound has no significant effect on either percent adult emergence or mean time to emergence in male and female leafcutting bees.

Table XIV. Percent emergence and emergence time (days) in control untreated and NX93 treated bee cells.

TREATMENT	EMERGENCE (%)	TIME TO EMERGENCE (DAYS) male	female	combined
Control	85.0%	20.68	21.41	20.90
NX93 (1%)	87.0%	20.73	22.43	21.40
NX93 (5%)	87.0%	21.08	22.13	21.65
NX93 (10%)	89.0%	20.98	21.80	21.28

3. Chalkbrood Disease Control Research

a) Paraformaldehyde fumigation of leafcutting bee cells

Research on the efficacy of paraformaldehyde fumigation for control of leafcutting bee-related moulds has shown that under specific temperature and humidity conditions (20-25°C/60-70%RH), fumigation of leafcutting bee nest material with paraformaldehyde at a rate of $20g/m^3$ will give total control of microorganisms in contaminated nest material.

Another area of leafcutting bee management where contamination is a problem is on bee cells. Research has indicated that the surfaces of these cells are inhabited by a wide range of mould, yeast, and bacterial species (microflora). This microflora may adhere to the body of the adult bee upon emergence from the cell and thus be transferred into nest material, causing spoilage of provisions and bee mortality. In paraformaldehyde fumigation tests on bee cells, evaluation of control and fumigated cell samples has indicated significant control of moulds (90.4-100.0%), yeasts (99.9-100.0%), and bacteria (94.3-100.0%) on cell surfaces (Table XV).

The moulds Aspergillus niger and Rhizopus oryzae, along with most yeast and bacterial species, were almost completely eliminated. Only Penicillium species were identified in cultured samples taken from treated cells and these were present in relatively low numbers. No significant differences were observed in either percent emergence or time to emergence of adult bees in incubation tests of control and treated cells.

Table XV. Efficacy of the fumigant paraformaldehyde at a rate of 20g/m³ for control of microflora on the surface of leafcutting bee cells under field conditions.

LOCATION	MICROORGANISM	NO. CFU PRE-TREAT.	NO. CFU POST-TREAT.	% CONTROL
Site 01	Moulds	1066	0	100.0
	Yeasts	7850	0	100.0
	Bacteria	1101	0	100.0
	Total	10017	0	100.0
Site 02	Moulds	1238	119	90.4
	Yeasts	7050	12	99.8
	Bacteria	3025	173	94.3
	Total	11313	304	97.3

In combined tests, mean time to adult emergence was 29.1 days and 28.4 days in control and treated groups, respectively; percent adult emergence was 93.9% (control) and 94.9% (treated). A summary of data from experiments conducted on control of microflora (combined mould, yeast, and bacterial species) on bee cell surfaces is given in Table XVI.

Table XVI. Efficacy of paraformaldehyde fumigation (20.0g/m³) for control of microflora on leafcutting bee cell surfaces.

MATERIAL TREATED		n	MEAN CFU	RANGE MIN.	RANGE MAX.	(SE)	% CFU REDUCTION
Bee Cells	Control	24	888.8	175	2155	133.0	-
Bee Cells	Treated	24	12.8	0	232	9.6	98.6[a]

[a] Reduction of combined microflora significant at $p<0.001$ level.

Paraformaldehyde fumigation for control of microflora on bee cell surfaces has been shown to be highly efficacious; an amendment of the current paraformaldehyde registration (originally obtained in March, 1992 for fumigation of nest material) to include bee cell fumigation was requested in April, 1993 from the Plant Industry Directorate, Agriculture Canada, Ottawa. This amendment request, supported by project research data on bee cell fumigation, was approved in September, 1993. An extension bulletin on paraformaldehyde fumigation of alfalfa leafcutting bee cells for control of microflora including chalkbrood was published in October, 1993, along with a revised paraformaldehyde label. Alfalfa seed producers will thus be able to utilize paraformaldehyde fumigation technology for treatment of both nest material and leafcutting bee cells prior to the upcoming 1994 field season.

b) Efficacy of paraformaldehyde for chalkbrood control

Experimentation has also been undertaken to assess the efficacy of paraformaldehyde fumigation for control of chalkbrood, a disease caused by the fungus Ascosphaera aggregata. Chalkbrood infects leafcutting bee larvae after they ingest spores of the fungus while feeding on pollen. Once the larvae die, millions of spores may form on the surface of the cadaver. The disease is currently the most serious limiting factor to leafcutting bee populations and alfalfa seed production in the northwestern United States. In Canada, chalkbrood has become a problem in southern Alberta, with levels as high as 25.6% in some populations. These widespread chalkbrood problems increase the probability of accidental introduction of the disease into areas where leafcutting bee populations are not yet infected with chalkbrood, such as Saskatchewan and Manitoba.

In order to conduct research in an area where chalkbrood was present at levels required for experimental purposes, a collaborative research program was undertaken with research personnel at the Agriculture Canada Research Station in Lethbridge, Alberta. In trials to assess paraformaldehyde efficacy, A. aggregata spores were placed inside plastic straws cut to fit inside tunnels of laminated polystyrene and wood nest material. These straws were then randomly placed in nest tunnels prior to paraformaldehyde fumigation. Following fumigation, the germination and viability of spores was determined in the laboratory.

Initial tests (series I) were undertaken in a cinder block building (3.6m x 3.2m x 3.1m) and involved four separate fumigations of 32 wood nest boxes each. The first fumigation was carried out according to label recommendations (i.e. 20g/m^3 @ 20-25°C, 60-70% R.H.), the second at a lower temperature than recommended, and the third and fourth tests at a lower relative humidity than recommended. Two large-scale commercial fumigation experiments (series II) were also undertaken, using a modified, insulated semi-trailer unit 2.4m x 13.1m x 2.5m. Each fumigation consisted of 497 nest boxes loaded on seven pallets.

In the first fumigation, polystyrene laminate nest material was fumigated at the recommended rate under higher than recommended temperature conditions, while in the second fumigation, wood laminate nest material was treated under similar conditions. In both tests, relative humidity was held at or above the recommended level. The results of the small- and large-scale tests (series I and II) are given below in Table XVII.

As the results indicate, in first two series I tests undertaken, 100% control of chalkbrood spores was achieved as spores from all tunnels showed no germination or viability. However, in the third and fourth series I tests, the lower humidity exerted a negative effect on the fumigation process, and control was variable in these two tests (20-99% and 21-94% respectively).

Table XVII. Efficacy of paraformaldehyde fumigation for control of chalkbrood spores in leafcutting bee nest material.

TEST	NEST MATERIAL TYPE	RELATIVE HUMIDITY (%)	TEMP. RANGE (°C)	CB SPORE CONTROL (%)
Series I				
01	WOOD / 10cm	74	16 - 23	100
02	WOOD / 10-15cm	75	9 - 12	100
03	WOOD / 15cm	45	16 - 26	20 - 99
04	WOOD / 15cm	42	21 - 24	21 - 94
Series II				
01	WOOD / 9cm	59	32 - 38	100
02	POLY / 9cm	75	35 - 40	100

The first large-scale fumigation (Series II) again resulted in 100% control of chalkbrood spores in wood nest material and total control was also achieved in the second large-scale fumigation of polystyrene nest material. These results indicate that under recommended temperature and humidity conditions, fumigation with paraformaldehyde is a highly efficacious method for decontaminating alfalfa leafcutting bee nest material infected with chalkbrood spores. It appears that relative humidity is the most critical factor in obtaining successful chalkbrood control, since the process is clearly efficacious at a range of temperatures.

IV. Impact on Saskatchewan Agri-food Industry

Saskatchewan leafcutting bee and trapnest surveys undertaken in 1992 and 1993 have provided alfalfa seed producers with information on the status of chalkbrood disease, parasites, and other important categories (i.e. pollen and larval moulds, dead larvae, bee live count) in alfalfa leafcutting bee populations in the province. When a significant outbreak of chalkbrood disease was identified in a population in the northeast part of the province, the producer involved was immediately advised on procedures to prevent the spread of the disease and to assist in its eradication. All nest material and bee cells from the infested population were fumigated with paraformaldehyde prior to the 1993 field season, utilizing protocols from technology developed through research undertaken as part of this project. As well, field shelters were sprayed with a bleach solution as part of the clean-up procedure.

In late May, 1993 meetings with alfalfa seed producers were held in Tisdale, Carrot River, and Hudson Bay in order to transfer technology on chalkbrood control. The chalkbrood-infested population in the northeast was monitored through the 1993 field season with trapnests placed in shelters and at other field sites. Data from the 1993/94 Saskatchewan alfalfa leafcutting bee survey indicate that the level of chalkbrood infestation in the affected population has been significantly reduced through use of paraformaldehyde technology.

Alternative methods for parasite control have been evaluated and the efficacy of a pyrethrin-based aerosol (DS) and one other compound (NX93) for control of re-parasitism has been demonstrated. These compounds have also been shown to be safe to developing and emerging leafcutting bees. Alfalfa seed producers in the province have been informed of the hazards associated with the use of dichlorvos for parasite control. Project research has indicated that dichlorvos rate, treatment time, number of bees treated, and position of bees in the incubation tray may all significantly affect bee emergence. Relative humidity is an additional factor which may play a crucial role in dichlorvos-induced mortality in developing leafcutting bees. Work to develop a compound which may replace dichlorvos for parasite control in leafcutting bee populations is continuing with the cooperation of alfalfa seed producers in Saskatchewan.

The presence of microflora in leafcutting bee populations spoils nectar and pollen provisions and contributes to bee mortality. Many of the mould species found in leafcutting bee populations are not only detrimental to the bees, but also pose a potential health hazard to alfalfa seed producers. Leafcutting bee incubation and harvesting operations can lead to high levels of airborne spores from many mould species. For these reasons, incorporation of control techniques is essential to reduce levels of microflora in the leafcutting bee environment.

Research with the fumigant paraformaldehyde has demonstrated that it is highly efficaceous for control of leafcutting bee microflora, including chalkbrood, which are found on the surfaces of bee cells. In response to the chalkbrood infestation in northeast Saskatchewan, an amendment of the current paraformaldehyde registration (originally obtained in March, 1992 for fumigation of nest material) to include bee cell fumigation was requested in April, 1993 from the Plant Industry Directorate, Agriculture Canada, Ottawa. This amendment request, supported by project research data on bee cell fumigation and a modified paraformaldehyde label, was approved on September 16th, 1993. An extension bulletin on paraformaldehyde fumigation of alfalfa leafcutting bee cells for control of microflora including chalkbrood was subsequently published. Alfalfa seed producers will thus be able to utilize paraformaldehyde fumigation technology for treatment of both nest material and leafcutting bee cells prior to the upcoming 1994 field season.

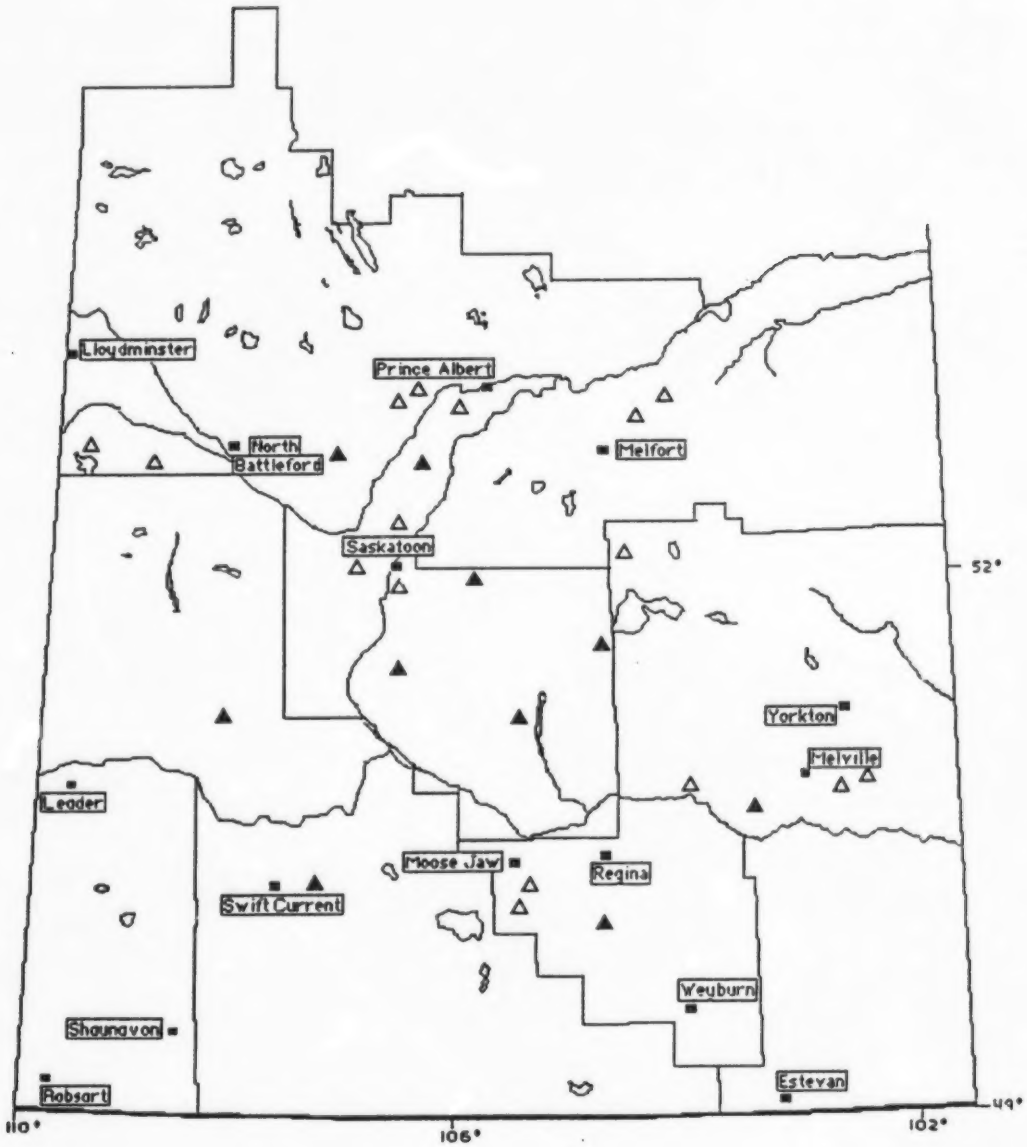
Surveys to accurately determine levels of parasites and chalkbrood disease in Saskatchewan, along with research to develop new methods for controlling parasites and microflora in Saskatchewan alfalfa leafcutting bee populations, serve to beneficially impact alfalfa seed producers by making them aware of the extent of these problems in their leafcutting bee populations and by transferring to them new technology to control these problems. More comprehensive utilization of parasite control and decontamination techniques will result in a reduction in levels of parasites and microflora in bee populations and will therefore increase population viability. This will enhance alfalfa seed production in the province and also increase the value of Saskatchewan alfalfa leafcutting bees in the world export market.

Appendix A.

Table I. 1989-1993 Saskatchewan Leafcutting Bee Survey data (%).

CATEGORY	1989	1990	1991	1992	1993
HEALTHY PREPUPAE	79.4	80.1	79.2	73.1	70.6
DEAD LARVAE/PREPUPAE					
Discoloured	3.7	3.1	3.3	4.3	5.6
Mouldy	0.3	0.2	0.2	0.2	0.3
Ascosphaera aggregata	-	-	-	0.017	0.007
Non-spor. chalkbrood	0.009	-	0.003	0.031	0.004
Other *Ascosphaera* spp.	0.007	0.011	0.005	0.020	0.128
MOULDY COCOONS					
Pollen Mould	2.8	2.5	3.7	3.2	6.6
Pollen *Ascosphaera* spp.	0.6	1.1	1.2	1.5	2.5
PARASITES	1.8	1.1	1.8	0.4	0.1
SECOND GENERATION	0.9	0.6	0.4	0.3	0.2
POLLEN BALLS	8.9	8.7	8.0	13.0	10.9
CRUSHED COCOONS	1.5	2.2	1.9	3.7	2.6
OTHER	0.3	0.5	0.2	0.3	0.3
LIVE COUNT/LB.	4089	4164	4232	3859	4069
LIVE COUNT/KG.	9004	9170	9316	8495	8966
NO. OF SAMPLES	76	55	61	84	97

Appendix B.



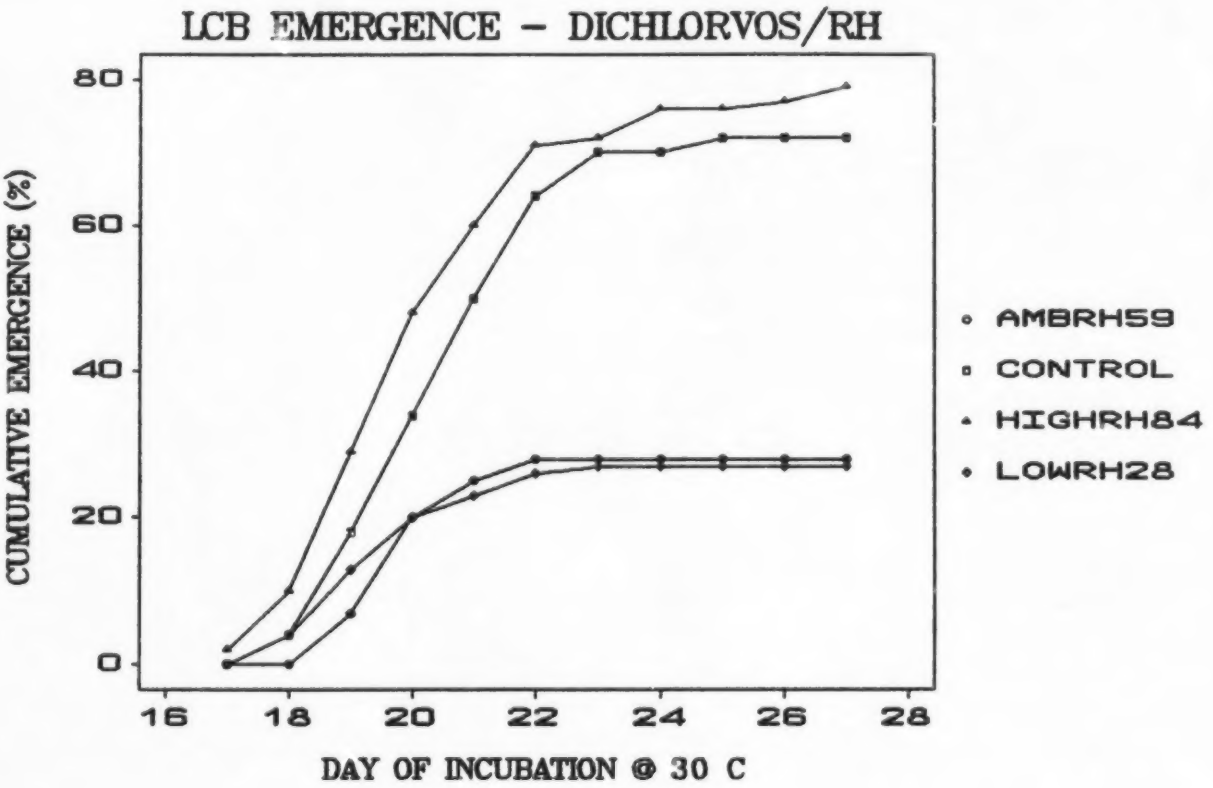
1992/1993 SASKATCHEWAN TRAPNEST LOCATIONS

▲ Primary Trap Nest Sites (4 nests)

△ Secondary Trap Nest Sites (1 nest)

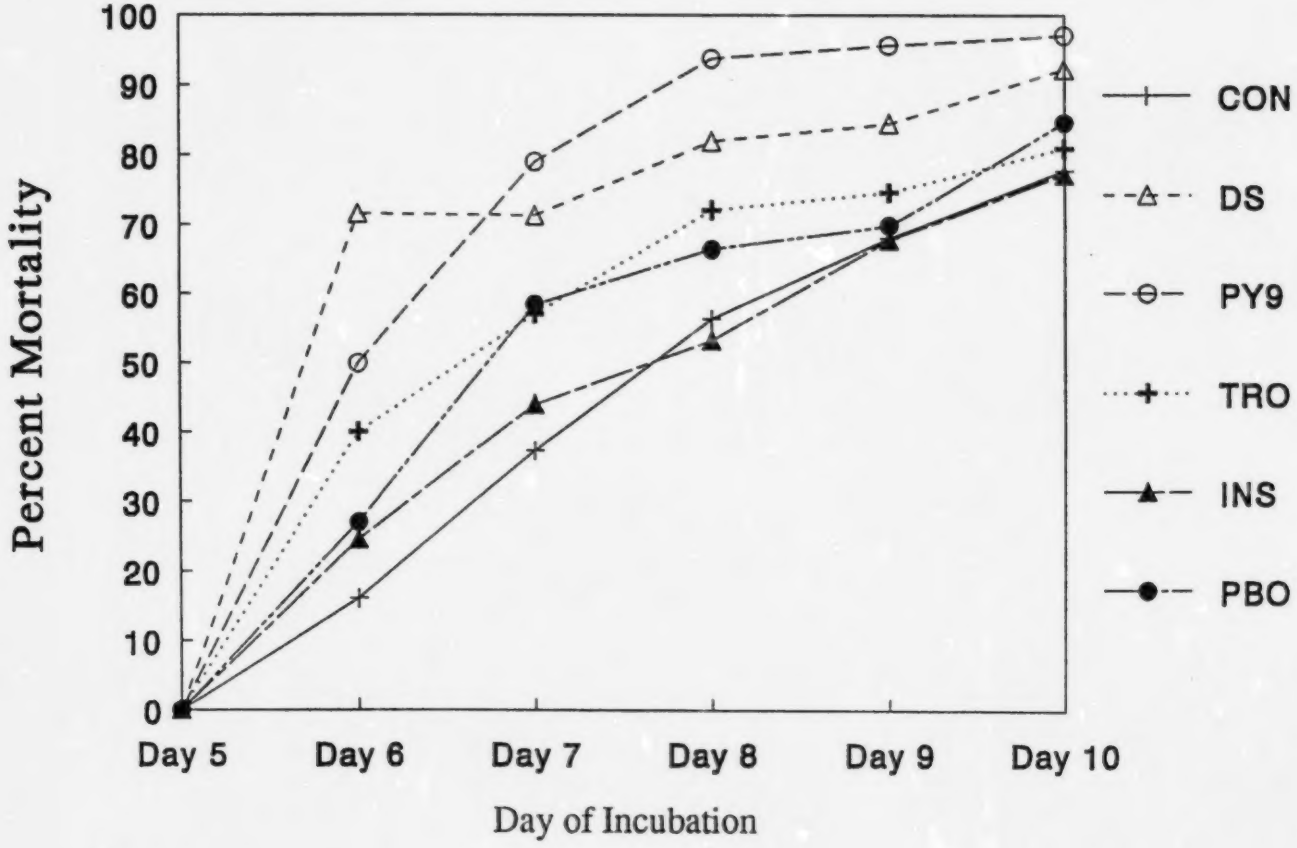
Appendix C.

Emergence in alfalfa leafcutting bees treated with dichlorvos at 1x the recommended rate (0.75 resin strip/1000 ft^3) under low (28%), ambient (59%) and high (84%) relative humidity conditions.



Appendix D.

Percent Mortality in P. venustus



C. Project Achievements / Technology Transfer

I. Project Achievements (1992-1994)

1. Research has demonstrated that paraformaldehyde fumigation of alfalfa leafcutting bee nest material, technology developed as part of this project, is highly efficacious for control of chalkbrood, a disease which has devastated alfalfa leafcutting bee populations in many alfalfa seed producing areas of North America. Utilization of this technology by seed producers in the province will help to keep Saskatchewan leafcutting bee populations disease-free, a factor of great importance to the leafcutting bee export market.

2. A request to add fumigation of leafcutting bee cells to the current paraformaldehyde label, which permits fumigation of leafcutting bee nest material, has been approved by the Plant Industry Directorate, Agriculture Canada. This amendment request was supported by research data from this project. The amended registration for paraformaldehyde was granted effective September 16th, 1993.

3. Two potential candidates for leafcutting bee parasite control have been identified. Tests carried out to date have demonstrated that treatment of leafcutting bee cells with compounds designated DS and NX93 will significantly reduce re-parasitism of the cells by the chalcid parasite, Pteromalus venustus.

II. Technology Transfer (1992-1994)

1. Extension Publications

Goerzen, D.W. Paraformaldehyde fumigation of alfalfa leafcutting bee nest material. Sask. Agriculture Development Fund Ext. Bulletin (ISBN 0-88656-556-1), 2pp. February, 1992.

Goerzen, D.W. Alfalfa leafcutting bee moulds and methods for their control. Sask. Agriculture Development Fund Ext. Bulletin (ISBN 0-88656-586-3), 4pp. March,1992.

Goerzen, D.W. Paraformaldehyde fumigation of alfalfa leafcutting bee nest material. Research Letter No. 247, 1 p. April, 1992. Agriculture Canada Research Branch, Research Station, Saskatoon.

Goettel, M.S. and D.W. Goerzen. Fumigant protects leafcutting bees from disease. Weekly Letter No. 3053, 1 p. November, 1992. Agriculture Canada Research Branch, Research Station, Lethbridge.

Goerzen, D.W. Paraformaldehyde fumigation for control of microflora including chalkbrood on the surface of alfalfa leafcutting bee cells. Sask. Alfalfa Seed Producers Association Misc. Publ., 1p. (May, 1993).

Goerzen, D.W. Paraformaldehyde fumigation for surface decontamination of alfalfa leafcutting bee cells. Sask. Alfalfa Seed Producers Association Extension Bulletin No. 93-01 (ISBN 0-88656-577-4), 2pp. (October, 1993).

Murrell, D.C. and D.W. Goerzen. Control of chalkbrood in alfalfa leafcutting bees. Sask. Agriculture and Food Extension Bulletin (ISBN 0-88656-598-7), 4pp. (April, 1994 - IN PRESS).

2. Publications in Refereed Journals

Goerzen, D.W., L. Dumouchel, and J. Bissett. 1992. Occurrence of chalkbrood disease, Ascosphaera aggregata Skou, in a native leafcutting bee, Megachile pugnata Say (Hymenoptera: Megachilidae). The Canadian Entomologist 124: 557-558.

Goerzen, D.W. and D.C. Murrell. 1992. Effect of fall dichlorvos treatment for control of a chalcid parasitoid on viability of the alfalfa leafcutting bee, Megachile rotundata (Fabricius) (Hymenoptera: Megachilidae). Bee Science 2: 37-42.

Goerzen, D.W., K.W. Richards, and G.H. Rank. 1994. Drifting in populations of the alfalfa leafcutting bee, Megachile rotundata (Fab.) (Hymenoptera: Megachilidae). (J. Appl. Entomol. - IN REVIEW).

Goettel, M.S., K.W. Richards, and D.W. Goerzen. 1993. Control of Ascosphaera aggregata spores in alfalfa leafcutting bee (Megachile rotundata) nesting materials by fumigation with paraformaldehyde. Bee Science 3(1): 22-25.

3. Papers in Published Conference Proceedings

Goerzen, D.W. 1992. Parasite control methods and related effects on leafcutting bee viability, and a summary of research on paraformaldehyde as a fumigant for control of leafcutting bee microflora. Proceedings of the 11th Canadian Alfalfa and Forage Seed Conference, Winnipeg, Manitoba. pp. 35-42.

Goerzen, D.W. 1992. Paraformaldehyde for fumigation of leafcutting bee nest material and research on parasite control methods and leafcutting bee viability. Proceedings of the 12th Seminar on Alfalfa Seed Production in the Peace River Region, Fairview, Alberta. Publ. 92-03, pp. 29-36.

Goerzen, D.W. 1992. Microflora associated with the alfalfa leafcutting bee (Megachile rotundata) and new technology for control of leafcutting bee microflora. Proceedings of the International Symposium on non-Apis Bees and their Role as Crop Pollinators, Logan, Utah. p.23.

Goerzen, D.W. 1993. A synopsis of alfalfa leafcutting bee research currently underway in western Canada. Proceedings of the 1st International Alfalfa Seed Conference / 24th Northwest Alfalfa Seed Conference, Reno, Nevada. pp. 1-3.

Goerzen, D.W. 1993. Paraformaldehyde fumigation research for mould and chalkbrood control and development of alternative methods for control of parasites in alfalfa leafcutting bee populations. Proceedings of the 1st International Alfalfa Seed Conference / 24th Northwest Alfalfa Seed Conference, Reno, Nevada, pp. 5-12.

Goerzen, D.W. 1993. Saskatchewan trapnest project update and status of chalkbrood in Saskatchewan. Proceedings of the 13th Seminar on Alfalfa Seed Production in the Peace River Region, Fairview, Alberta. Publ. 93-01, pp. 30-31.

Goerzen, D.W. 1994. Current research on leafcutting bee parasite control and an update on paraformaldehyde fumigation for control of bee microflora. Proceedings of the 12th Canadian Alfalfa and Forage Seed Conference, Edmonton, Alberta. pp. 13-18.

Goerzen, D.W. 1994. Parasite control research and a new amendment to the registration of paraformaldehyde for control of microflora including chalkbrood. Proceedings of the 25th Northwest Alfalfa Seed Conference, Reno, Nevada. pp. 36-41.

Goerzen, D.W. 1994. Evaluation of two potential parasite control compounds and efficacy of paraformaldehyde fumigation for control of microflora, including chalkbrood, on bee cell surfaces. Proceedings of the 14th Seminar on Alfalfa Seed Production in the Peace River Region, Fairview, Alberta. Publ. 94-03, pp. 20-25.

Murrell, D.C. and D.W. Goerzen. 1992. Fall dichlorvos treatment for parasite control. Proceedings of the 23rd Northwest Alfalfa Seed Conference, Boise, Idaho. pp. 11-15.

4. Contributed Research Abstracts

Goerzen, D.W. 1992. Microflora associated with the alfalfa leafcutting bee in Saskatchewan. Research Highlights 1991, Agriculture Canada Research Station, Saskatoon. p. 34.

Goerzen, D.W. 1992. Status of parasites and chalkbrood in Saskatchewan alfalfa leafcutting bee populations. Minutes of the Western Committee on Crop Pests (Sask. Res. Report).

Goerzen, D.W. 1992. Status of alfalfa leafcutting bee populations. Report of the Diseases and Insects Subcommittee, Sask. Advisory Committee on Forage Crops.

Goerzen, D.W. and T.C. Watts. 1992. Paraformaldehyde fumigation for control of microflora in alfalfa leafcutting bee populations. Research Highlights 1991, Agriculture Canada Research Station, Saskatoon. pp. 34-35.

Goerzen, D.W. 1993. Status of parasites and chalkbrood in Saskatchewan alfalfa leafcutting bee populations. Minutes of the Western Committee on Crop Pests (Sask. Res. Report).

Goerzen, D.W. 1993. Status of alfalfa leafcutting bee populations. Report of the Diseases and Pests Subcommittee, Sask. Advisory Committee on Forage Crops.

Goettel, M.S., K.W. Richards, and D.W. Goerzen. 1993. Efficacy of paraformaldehyde fumigation for decontamination of Ascosphaera aggregata spores from alfalfa leafcutting bee Megachile rotundata nest materials. Abstr. of the Society for Invertebrate Pathology XXVIth Annual Meeting, Asheville, North Carolina. p.28.

5. Conference Presentations

a) 11th Canadian Alfalfa and Forage Seed Conference. January 9-11th, 1992; Winnipeg, Manitoba.

b) 12th Annual Seminar on Alfalfa Seed Production
in the Peace River Region.
March 19-20th, 1992;
Fairview, Alberta.

c) International Symposium on non-Apis Bees
and their Role as Crop Pollinators
August 10-13th, 1992;
Logan, Utah.

d) 1st International Alfalfa Seed Conference
January 24-27th, 1993;
Reno, Nevada.

e) 12th Canadian Alfalfa and Forage Seed Conference.
January 6-8th, 1994;
Edmonton, Alberta.

6. Presentations to Producer Groups

a) 20th Saskatchewan Alfalfa Seed Producers
Association Annual Meeting, January 6-7th, 1992;
Saskatoon, Saskatchewan.

b) Irrigated Alfalfa Seed Producers
Annual Meeting, March 26th, 1992;
Brooks, Alberta.

c) 1992 Saskatchewan Alfalfa Seed Producers
Association Field Day, July 16th, 1992;
Parkside, Saskatchewan.

d) 21st Saskatchewan Alfalfa Seed Producers
Association Annual Meeting, January 4-5th, 1993;
Saskatoon, Saskatchewan.

e) Manitoba Forage Seed Association
Annual Meeting, January 7-9th, 1993;
Winnipeg, Manitoba.

f) Irrigated Alfalfa Seed Producers
Annual Meeting, March 16th, 1993;
Brooks, Alberta.

g) Alfalfa Seed Producers (NE Saskatchewan)
Chalkbrood Information / Control Strategy Meeting,
May 27th, 1993;
Tisdale, Saskatchewan.

h) 1993 Saskatchewan Alfalfa Seed Producers Association Field Day, July 22nd, 1993; Melfort / St. Brieux, Saskatchewan.

i) 22nd Saskatchewan Alfalfa Seed Producers Association Annual Meeting, January 3-4th, 1994; Saskatoon, Saskatchewan.

j) Manitoba Forage Seed Association Annual Meeting, January 17-18th, 1994; Winnipeg, Manitoba.

k) Montana Alfalfa Seed Growers Association Annual Meeting, February 17th, 1994; Billings, Montana.

7. Newsletter Contributions

(1992/1993)

a) SASPA Newsletter, Vol.5, No.1. Parasite control methods and their effects on leafcutting bee viability, a summary of research on paraformaldehyde for fumigation of leafcutting bee nest material, and an update on the status of chalkbrood in Saskatchewan. February, 1992; pp.21-30.

b) Sask. Agriculture and Food / Forage Seed News, No.1. Paraformaldehyde fumigation of alfalfa leafcutting bee nest material. March, 1992; appendix 1.

c) Alberta Irrigated Alfalfa Seed Producers Winter Newsletter. Parasite control methods and related effects on leafcutting bee viability, and a summary of research on paraformaldehyde as a fumigant for control of leafcutting bee microflora. March, 1992; pp.1-5.

d) Ibid. Fumigating alfalfa leafcutting bee nesting materials for control of chalkbrood disease. March, 1992; pp.25-28.

e) Ibid. Efficacy of the fumigant paraformaldehyde for control of microflora associated with the alfalfa leafcutting bee. March, 1992; pp.29-35.

f) IMP Alfalfa Seed / Washington State Cooperative Extension Newsletter, Vol.5, No.4. Paraformaldehyde fumigation of leafcutting bee nests. April, 1992; p.1.

g) Alberta Irrigated Alfalfa Seed Producers Spring Newsletter. Paraformaldehyde fumigation of alfalfa leafcutting bee nest material. May, 1992; pp.9-11.

h) Manitoba Agriculture / Forage Seed Update. Paraformaldehyde fumigation of alfalfa leafcutting bee nest material. May, 1992; pp.29-31.

i) Sask. Agriculture and Food / Forage Seed News, No.2. 1991/92 Saskatchewan leafcutting bee survey data and an evaluation of chalcid parasite levels (1985-1991). June, 1992; pp. 19-22.

j) SASPA Newsletter, Vol.5, No.2. Saskatchewan leafcutting bee survey data and an evaluation of chalcid parasite levels (1985-1991). June, 1992; pp.3-6.

k) Sask. Agriculture and Food / Forage Seed News, No.3. Research on alternative methods for control of parasites of the alfalfa leafcutting bee. October, 1992; pp.14-17.

l) SASPA Newsletter, Vol.5, No.3. 1992 Saskatchewan trapnest update and status of chalkbrood in Saskatchewan. November, 1992; pp.3-4.

m) Alberta Irrigated Alfalfa Seed Producers Fall Newsletter. Research on alternative methods for control of parasites of the alfalfa leafcutting bee. November, 1992; pp.24-27.

n) Manitoba Agriculture / Forage Seed Update. Research on alternative methods for control of parasites of the alfalfa leafcutting bee. November, 1992; pp.15-18.

o) Northwest Alfalfa Seed Newsletter. Canadian research: chalkbrood. Winter, 1992; p.9. Northwest U.S. Alfalfa Seed Producers.

p) SASPA Newsletter, Vol.6, No.1. Research on paraformaldehyde fumigation for control of alfalfa leafcutting bee microflora including chalkbrood, and research on alternative parasite control methods. February, 1993; pp.23-30.

q) Ibid. Paraformaldehyde fumigation of alfalfa leafcutting bee nesting materials for control of chalkbrood. February, 1993; pp.45-49.

r) Alberta Irrigated Alfalfa Seed Producers Winter Newsletter. Paraformaldehyde fumigation of alfalfa leafcutting bee nest material. February, 1993; pp.35-38.

s) Manitoba Agriculture / Forage Seed Update. A research update on paraformaldehyde fumigation for mould and chalkbrood control, and the development of alternative methods for parasite control. March,1993; pp.8-15.

t) The Manitoba Co-operator. Fumigant helps to reduce mould in leafcutting bee nests. March 18th, 1993; p.27.

(1993/1994)

a) Manitoba Agriculture / Forage Seed Update. Paraformaldehyde fumigation of alfalfa leafcutting bee nesting materials for control of chalkbrood. May, 1993; pp.8-12.

b) Ibid. Paraformaldehyde fumigation of alfalfa leafcutting bee nest materials. May, 1993; pp.23-25.

c) Sask. Agriculture and Food / Forage Seed News. 1992/1993 Saskatchewan leafcutting bee survey data and a summary of chalcid parasite levels (1985-1992). May, 1993; pp.8-11.

d) Ibid. Paraformaldehyde fumigation of alfalfa leafcutting bee nesting materials for control of chalkbrood. pp.21-25.

e) Alberta Irrigated Alfalfa Seed Producers Spring Newsletter. Paraformaldehyde fumigation of alfalfa leafcutting bee nesting materials for control of chalkbrood. June, 1993; pp.12-16.

f) SASPA Newsletter, Vol.6, No.2. Request for amended registration of paraformaldehyde for fumigation of alfalfa leafcutting bee cells. June, 1993; p.2.

g) Ibid. 1992/1993 Saskatchewan leafcutting bee survey data and a summary of chalcid parasite levels (1985-1992). pp.3-6.

h) Northwest Alfalfa Seed Growers Association Newsletter. Saskatchewan report (bee survey). Summer, 1993; pp.11-14.

i) Manitoba Agriculture / Forage Seed Update. 1993 parasite control research project update. October, 1993; pp.9-12.

j) SASPA Newsletter, Vol.6 No.3. 1993 Saskatchewan trapnest survey report and status of chalkbroodin Saskatchewan. November, 1993; pp.6-7.

k) Ibid. 1993 parasite control research project update. November, 1993; pp.8-11.

l) Ibid. Report on research to study the potential for using the alfalfa leafcutting bee for pollination of almond in central California. November, 1993; pp.12-13.

m) Alberta Irrigated Alfalfa Seed Producers Fall Newsletter. 1993 parasite control research project update. November, 1993; pp.3-6.

n) Sask. Agriculture and Food / Forage Seed News. Development of alternative technology for control of chalcid parasites in alfalfa leafcutting bee populations - 1993 research update. November, 1993; pp.19-22.

o) Ibid. Paraformaldehyde fumigation for surface decontamination of alfalfa leafcutting bee cells. November, 1993; pp.23-25.

p) Alberta Irrigated Alfalfa Seed Producers Winter Newsletter. Current research on leafcutting bee parasite control and an update on paraformaldehyde fumigation for control of bee microflora. February, 1994; pp.3-7.

q) SASPA Newsletter, Vol.7, No.1. Research on two new candidate compounds for chalcid paracite control and efficacy of paraformaldehyde fumigation for control of microflora on bee cell surfaces. February, 1994; pp.23-28.

r) Manitoba Agriculture / Forage Seed Update. Paraformaldehyde fumigation for surface decontamination of alfalfa leafcutting bee cells. March, 1994; pp.15-17.

D. Personnel

Research Personnel

D.W. Goerzen, Biologist - 100% of time working on project.

Producer Cooperators

L. Anderson, Parkside
G. Bacsu, Tisdale
B. Boan, Moose Jaw
B. Dowling, Macdowall
H. Dutka, Saskatoon
P. Eggerman, Watson
T. Fortier, Zenon Park
B. Gourley, Watson
K. Hansen, Marsden
R. Herperger, Atwater
W. Mennie, Parkside
M. Pask, Atwater
D. Swenson, Moose Jaw
E. Tanner, Indian Head
L. Veikle, Cutknife
B. Wolf, Carrot River

E. Equipment

No capital equipment was purchased for ADF Project R-92-CA-0739 during the period April 01, 1992 through March 31, 1994.

G. ADF Acknowledgement

The partial support of the Saskatchewan Agriculture Development Fund for the research project entitled "Assessment of parasites and chalkbrood disease in Saskatchewan alfalfa leafcutting bee populations and development of control methods" was acknowledged in all extension bulletins, scientific papers, contributions to conference proceedings, and newsletter articles pertaining to the project and published during the period April 01, 1992 through March 31, 1994. This support was also acknowledged in all presentations to producer groups and conference presentations pertaining to the project.